

Marttila, M. et al. (2014) Mutation update and genotype-phenotype correlations of novel and previously described mutations in TPM2 and TPM3 causing congenital myopathies. *Human Mutation*, 35(7), pp. 779-790.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

<http://eprints.gla.ac.uk/122383/>

Deposited on: 09 August 2016

Published in final edited form as:

Hum Mutat. 2014 July ; 35(7): 779–790. doi:10.1002/humu.22554.

Mutation Update and Genotype–Phenotype Correlations of Novel and Previously Described Mutations in *TPM2* and *TPM3* Causing Congenital Myopathies

Minttu Marttila¹, Vilma-Lotta Lehtokari¹, Steven Marston², Tuula A. Nyman³, Christine Barnerias⁴, Alan H. Beggs⁵, Enrico Bertini⁶, OÖzge Ceyhan-Birsoy⁵, Pascal Cintas⁷, Marion Gerard⁸, Brigitte Gilbert-Dussardier⁹, Jacob S. Hogue¹⁰, Cheryl Longman¹¹, Bruno Eymard¹², Moshe Frydman¹³, Peter B. Kang¹⁴, Lars Klinge¹⁵, Hanna Kolski¹⁶, Hans Lochmüller¹⁷, Laurent Magy¹⁸, Véronique Manel¹⁹, Michèle Mayer²⁰, Eugenio Mercuri²¹, Kathryn N. North²², Sylviane Peudenier-Robert²³, Helena Pihko²⁴, Frank J. Probst²⁵, Ricardo Reisin²⁶, Willie Stewart²⁷, Ana Lia Taratuto²⁸, Marianne de Visser²⁹, Ekkehard Wilichowski³⁰, John Winer³¹, Kristen Nowak³², Nigel G. Laing³², Tom L. Winder³³, Nicole Monnier³⁴, Nigel F. Clarke³⁵, Katarina Pelin³⁶, Mikaela Grönholm³⁷, and Carina Wallgren-Pettersson^{1,*}

¹The Folkhälsan Institute of Genetics and the Department of Medical Genetics, University of Helsinki, Haartman Institute, Biomedicum Helsinki, Finland ²National Heart and Lung Institute, Imperial College London, London, UK ³Institute of Biotechnology, University of Helsinki, Helsinki, Finland ⁴Cliniques des maladies du développement CHU Paris, Paris, France ⁵Division of Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts ⁶Unit of Neuromuscular and Neurodegenerative Disorders, Bambino Gesù' Children's Research Hospital, Rome, Italy ⁷Centre de référence de pathologie neuromusculaire, CHU Toulouse, Toulouse, France ⁸Service de Génétique, CHU Clémenceau, Caen, France ⁹Genetics, University Hospital La Milettrie, Poitiers, France ¹⁰The Department of Pediatrics, San Antonio Military Medical Center, Houston, Texas ¹¹West of Scotland Regional Genetics Service, Glasgow, UK ¹²Centre de Référence de pathologie neuromusculaire Paris-Est, Groupe Hospitalier Pitié-Salpêtrière, Paris, France ¹³Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel Aviv University, Tel Aviv, Israel ¹⁴Department of Neurology, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts ¹⁵University Medical Centre Göttingen, Göttingen, Germany ¹⁶Glenrose Rehabilitation Hospital, University of Alberta, Alberta, Canada ¹⁷Institute of Genetic Medicine, Newcastle University, Tyne and Wear, UK ¹⁸National Referral Center for Rare Peripheral Neuropathies, Centre Hospitalier Universitaire, Limoges, France ¹⁹Centre de Référence des Maladies Neuromusculaires Rhône-Alpes, Hôpital Femme-Mère-Enfant, Lyon, France ²⁰Centre de Référence et de Suivi des Maladies Neuromusculaires, Hôpital Trousseau, Paris, France

© 2014 Wiley Periodicals, Inc.

*Correspondence to: Carina Wallgren-Pettersson, The Folkhälsan Department of Medical Genetics, P.O. Box #211, Helsinki FIN-00251, Finland. carina.wallgren@helsinki.fi.

Additional Supporting Information may be found in the online version of this article.

Disclosure statement: The authors declare that there is no conflict of interest.

²¹Pediatric Neurology, Catholic University, Rome, Italy ²²Murdoch Children's Research Institute, Melbourne, Australia ²³Pôle de la Femme, de la Mère et de l'Enfant, Centre Hospitalier Régional Universitaire Brest, Brest, France ²⁴University of Helsinki Central Hospital, Helsinki, Finland ²⁵Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas ²⁶Hospital Británico de Buenos Aires, Buenos Aires, Argentina ²⁷Department of Pathology, Southern General Hospital, Glasgow, UK ²⁸Department of Neuropathology, Institute for Neurological Research, FLENI, Buenos Aires, Argentina ²⁹Department of Neurology, Academic Medical Center, Amsterdam, The Netherlands ³⁰Department of Pediatrics and Pediatric Neurology, Georg August University, Göttingen, Germany ³¹University Hospital Birmingham, Queen Elizabeth Hospital, Birmingham, UK ³²Molecular Neurogenetics Laboratory, Centre for Medical Research, University of Western Australia, Nedlands, Australia ³³PreventionGenetics, Marshfield, Massachusetts ³⁴Laboratoire de Biochimie et Génétique Moléculaire, IBP-CHU Grenoble, Grenoble, France ³⁵INMR, The Children's Hospital at Westmead and Sydney Medical School, University of Sydney, Sydney, Australia ³⁶Division of Genetics, Department of Biosciences, University of Helsinki, Helsinki, Finland ³⁷Division of Biochemistry and Biotechnology, Department of Biosciences, University of Helsinki, Finland

Abstract

Mutations affecting skeletal muscle isoforms of the tropomyosin genes may cause nemaline myopathy, cap myopathy, core-rod myopathy, congenital fiber-type disproportion, distal arthrogryposes, and Escobar syndrome. We correlate the clinical picture of these diseases with novel (19) and previously reported (31) mutations of the *TPM2* and *TPM3* genes. Included are altogether 93 families: 53 with *TPM2* mutations and 40 with *TPM3* mutations. Thirty distinct pathogenic variants of *TPM2* and 20 of *TPM3* have been published or listed in the Leiden Open Variant Database (<http://www.dmd.nl/>). Most are heterozygous changes associated with autosomal-dominant disease. Patients with *TPM2* mutations tended to present with milder symptoms than those with *TPM3* mutations, DA being present only in the *TPM2* group. Previous studies have shown that five of the mutations in *TPM2* and one in *TPM3* cause increased Ca^{2+} sensitivity resulting in a hypercontractile molecular phenotype. Patients with hypercontractile phenotype more often had contractures of the limb joints (18/19) and jaw (6/19) than those with nonhypercontractile ones (2/22 and 1/22), whereas patients with the non-hypercontractile molecular phenotype more often (19/22) had axial contractures than the hypercontractile group (7/19). Our in silico predictions show that most mutations affect tropomyosin–actin association or tropomyosin head-to-tail binding.

Keywords

congenital myopathy; genotype–phenotype correlation; *TPM2*; *TPM3*; actin; hypercontractile phenotype

Introduction

The congenital myopathies are clinically, histologically, and genetically variable neuromuscular disorders, often caused by mutations in genes encoding sarcomeric proteins [Wallgren-Pettersson et al., 2011]. These myopathies are defined on the basis of static or only slowly progressive muscle weakness and a range of structural abnormalities in the muscle fibers. In recent years, a number of different myopathy entities have been associated with mutations in one of the two tropomyosin genes, *TPM2* and *TPM3*. Nemaline myopathy (NM; MIMs #161800, #256030, #605355, #609273, #609284, #609285, #610687, #615348) is a genetically heterogeneous disorder showing wide clinical variability. It is characterized by muscle weakness and nemaline (rod) bodies on muscle biopsy [Wallgren-Pettersson et al., 2004]. NM was described by two separate groups in 1963 [Conen et al., 1963; Shy et al., 1963]. “Cap myopathy” or “cap disease” (MIMs #609284, #609285) was first characterized by Fidzianska et al. (1981). The name derives from the cap-like structures located under the sarcolemma. The first cases of congenital fiber-type disproportion (CFTD; MIM #255310) was described by Brooke (1973) in patients with hypotrophy of type 1 muscle fibers (Fig. 1). Arthrogryposis is a very heterogeneous disease category in which distal arthrogryposes (DA) is one subgroup of dominantly inherited disorders (Fig. 2). Daentl et al. (1974) described three patients with DA type 1A (MIM #108120). Another form, DA type 2B (MIM #601680) was first identified by Krakowiak et al. (1997). Escobar syndrome is a nonlethal type of multiple pterygium syndrome (MIM #265000). It was first characterized by Norum et al. (1969) and associated in one case with NM [Monnier et al., 2009]. Core-rod myopathy and autosomal-dominant trismus-pseudocamptodactyly syndrome (DA type 7) have also recently been associated with *TPM2* mutations [Davidson et al., 2013].

The roles of tropomyosins in stabilizing the thin (actin) filament of the sarcomere [Cooper, 2002] and in regulating muscle contraction [Gordon et al., 2000] have been well defined in skeletal muscle. The tropomyosins exist as coiled-coil homo- or hetero-dimers forming head-to-tail polymers, running along the length of the actin filament [Phillips et al., 1979; Matsumura et al., 1983; Holmes et al., 1990; Lin et al., 1997]. They are encoded by four different genes; *TPM1* (MIM # 191010), *TPM2* (MIM # 190990), *TPM3* (MIM # 191030), and *TPM4* (MIM # 600317) [Pittenger et al., 1994], generating more than 40 different tropomyosin isoforms due to the use of different promoters or variable intragenic splicing [Pittenger et al., 1994; Dufour et al., 1998; Cooley and Bergtrom, 2001]. The tropomyosin genes *TPM1*, *TPM2*, and *TPM3* encode the skeletal muscle isoforms Tm1 (α -tropomyosin_{fast}), Tm2 (β -tropomyosin), and Tm3 (α -tropomyosin_{slow}), respectively. *TPM1* is mainly expressed in fast muscle fibers and has lower expression in skeletal muscle than in cardiac muscle. *TPM2* is expressed in both slow, and, to a lesser extent, in fast muscle fibers. *TPM3* is expressed exclusively in slow muscle fibers. When both α - and β -tropomyosins are expressed, α , β -heterodimers are preferentially formed over α -homodimers, and β -homodimers are rare [Perry, 2001].

Here, we compare the nature and location of novel, and to the best of our knowledge, all previously described mutations of the *TPM2* and *TPM3* genes in patients with NM, CFTD, cap myopathy, core-rod myopathy, DA type 1A, DA type 2B, DA type 7, and NM with Escobar syndrome to explore the relationships between mutation type and position and the

phenotypes that arise. Ninety-three families were included in this study: 53 with *TPM2* mutations and 40 with *TPM3* mutations. Altogether 30 distinct pathogenic variants of *TPM2* and 20 of *TPM3* have been reported in the literature (including the 14 novel *TPM2* and 5 novel *TPM3* mutations, a total of 19 mutations reported for the first time in the current paper). We have also studied previously known and novel phosphorylation sites in tropomyosin with known patient mutations, and characterized the phosphorylation patterns in purified proteins containing known patient mutations compared with wild-type (wt) protein.

Families

Ascertainment

Families reported here for the first time were identified by neuromuscular services in different countries: Argentina, Australia, Canada, Finland, France, Germany, Israel, Italy, The Netherlands, UK, and USA. All patients had consented to research or diagnostic genetic testing. Institutional review board approval was obtained to access patient information, and written consent was obtained where appropriate.

TPM2

Fifty-three families had mutations of the *TPM2* gene. Patients in 13 of the families had been diagnosed with NM and in five families a diagnosis of cap myopathy was made. CFTD was the diagnosis in seven families. Ten families had a proband with DA. In one family, a member had NM with Escobar syndrome. NM and cap myopathy had both been diagnosed in three families. Affected members of two families with a diagnosis of NM also had significant fiber-type disproportion. Cap myopathy with fiber-type disproportion had been diagnosed in two families. One family had DA with unspecified congenital myopathy (CM) and one with core-rod myopathy. One proband had a diagnosis of CM and affected members of seven families had no specified diagnosis.

TPM3

Forty families had mutations in the *TPM3* gene. Patients in nine families had a diagnosis of NM and in 20 families, of CFTD with no specific histological hallmarks. Three probands had been diagnosed with cap myopathy. Two families had members with a diagnosis of NM and cap myopathy. Probands of four families had CM, and in two there was no specified diagnosis.

Mutations

Nomenclature of *TPM2* and *TPM3* Mutations and Database

For the description of sequence variants, we used reference sequence NM 003289.3 for *TPM2* and NM 152263.3 for *TPM3*. Amino acid coordinates are provided relative to NP 003280.2 and NP_689476.2, respectively. Note that *TPM3* mutations are numbered according to the Human Genome Variation Society recommendations [den Dunnen and Antonarakis, 2000], meaning the first two ATG codons in the primary cDNA were both included. *TPM2* and *TPM3* variants described in the article are listed in the Leiden Open

Variant Database (<http://www.dmd.nl/>) at the Center for Human and Clinical Genetics, Leiden University Medical Center.

Novel Mutations in *TPM2* and *TPM3*

We used Sanger sequencing and dHPLC to identify novel mutations in the *TPM2* and *TPM3* genes. Including previously published studies, 94 families were included in the study. Of these, 53 had *TPM2* mutations and 41 had *TPM3* mutations. There were 30 different mutations in *TPM2* (14 novel mutations we report and 16 previously described) and 20 different mutations in *TPM3* (five novel and 15 previously reported) (Tables 1 and 2; Fig. 3). No clinical details were available for 12 *TPM2* families and five *TPM3* families. None of these changes were found in the 1000 Genomes Dataset (www.1000genomes.org, accessed November 2013), nor in the Exome Variant Server, NHLBI Exome Sequencing Project (ESP) (Seattle, WA) (URL: <http://evs.gs.washington.edu/EVS/>, accessed November 2013).

Prediction of Pathogenicity of Mutations

PolyPhen-2, version 2.2.2, HumVar option (<http://genetics.bwh.harvard.edu/pph2/index.shtml>) [Adzhubei et al., 2010], and FATHMM (functional Analysis through hidden Markov models), version 2.3 with the prediction algorithm weighted for human mutations and disease ontology as the option for phenotypic associations (<http://fathmm.biocompute.org.uk>) [Shihab et al., 2013] were used to predict the functional effect of the missense mutations identified in *TPM2* and *TPM3*. Molecular modeling is described in detail by Memo and Marston (2013).

In *TPM2*, 17 of the 22 missense mutations were predicted to be possibly or probably damaging by PolyPhen-2, and FATHMM. However, there were slight discrepancies in the predictions by the two programs. PolyPhen-2 predicted three mutations (p.Asp2Val, p.Ala3Gly, and p.Arg91Gly) to be benign, whereas FATHMM predicted the same three mutations to be damaging. On the other hand, FATHMM predicted the mutation p.Gln147Pro to be tolerated, whereas PolyPhen-2 predicted the same mutation to be probably damaging. Both programs predicted two mutations (p.Glu41Lys and p.Asn202Lys) to be benign or tolerated (Table 1 and Supp. Tables S1 and S2). p.Glu41Lys has, however, been shown to affect muscle contractility due to decreased calcium sensitivity [Marttila et al., 2012], and p.Asn202Lys has been hypothesized to affect Tm–Troponin T (TnT) interactions [Ohlsson et al., 2008].

In *TPM3*, 16 of the 18 missense mutations were predicted to be possibly or probably damaging by PolyPhen-2, and 17 were predicted to be damaging by FATHMM. PolyPhen-2 predicted two mutations (p.Leu100Val and p.Arg168His) to be benign, whereas FATHMM predicted the same mutations to be damaging. On the other hand, FATHMM predicted the mutation p.Thr253Lys to be tolerated, whereas PolyPhen-2 predicted the same mutation to be possibly damaging (Table 2 and Supp. Tables S3, S4, and S5). Our analysis shows that both PolyPhen-2 and FATHMM were able to correctly predict more than 80% of the pathogenic Tm mutations (82.5% for PolyPhen-2 and 85% for FATHMM).

Recurrent Mutations in *TPM2* and *TPM3*

Two recurrent mutations were found in *TPM2*. The first is p.Lys7del, which was found in 10 families [Davidson et al., 2013; Mokbel et al., 2013; present study, see Table 1]. The second one is p.Glu139del, which has been reported in five families [Lehtokari et al., 2007; Clarke et al., 2009; Tasca et al., 2013; present study, see Table 1].

One mutational hotspot was identified in *TPM3*. The p.Arg168 residue was mutated in 21 families. The recurrent mutation p.Arg168His was present in 12 families [Durling et al., 2002; Penisson-Besnier et al., 2007; Clarke et al., 2008; De Paula et al., 2009; Lawlor et al., 2010; present study, see Table 2 and Supp. Table S6]. Eight families had the p.Arg168Cys mutation [Clarke et al., 2008; present study, see Table 2 and Supp. Table S6] and one family had the p.Arg168Gly mutation [Clarke et al., 2008; see Table 2.].

Clinical Relevance

Tropomyosins are actin-binding, coiled-coil proteins expressed in all eukaryotic cells. Their major function in skeletal muscle is to stabilize actin and to regulate actin–myosin interactions by limiting access to myosin-binding sites along the major groove of the actin filament [Gunning et al., 2005]. The tropomyosin α -helical coiled coil consists of heptapeptide repeats (*abcdefg*) where the *a* and *d* residues are generally nonpolar and form the interhelical interface (or core) of the double-stranded structure [Perry, 2001].

CM phenotypes can be classified into two types: those that are characterized by congenitally weak muscles and those with congenitally normal or hypercontractile muscles (e.g., DA). Contractility is the major factor that is altered by mutations in contractile proteins such as tropomyosin. The classification of these disorders is a challenge because a single mutation shared between patients can cause a range of phenotypes. This was seen in patients in two recently published papers on the Lys7del mutation [Davidson et al., 2013; Mokbel et al., 2013]. Within the 10 families described, the variability in phenotype is quite remarkable, complicating the recognition of any definite clinical and histological phenotype–genotype correlation.

NM, cap myopathy, and CFTD are closely related entities with largely overlapping clinical features, whereas DA and Escobar syndrome may be seen as more distinct clinical entities with less clinical overlap with other disorders within the group. Distal contractures are, however, seen in all these disease groups. The age at presentation did not appear to differ between the *TPM2* and *TPM3* groups. In both, the majority presented perinatally or in infancy, a smaller number in childhood, with delayed motor milestones, and few in adulthood.

Nine of the patients with *TPM2* mutations presented with distal arthrogryposis, whereas none of the patients with *TPM3* mutations presented with this feature. This may be due to Tm2 being expressed earlier during development than Tm3 [Muthuchamy et al., 1993]. Patients with the recurrent deletion mutation Lys7del in the 5'-end of the gene presented with jaw contractures, camptodactyly (2/10) or core-rod myopathy (1/10), contractures (4/10), and difficulty in opening the mouth (4/10) [Davidson et al., 2013; Mokbel et al., 2013]. Nemaline bodies were also present in patients with a diagnosis of DA type 7 (3/3),

exemplifying the overlap between phenotypic characteristics of these disease entities (Supp. Tables S1 and S2).

In most families in the present series, the disease-causing mutations were heterozygous, and the mode of inheritance dominant in 89 families. Of the dominant cases, 43 were due to de novo mutations. Few *TPM3* mutations were homozygous and these were all found in the beginning or at the end of the gene. Most recessive mutations were nonsense mutations changing an amino acid to a stop codon, yielding a truncated protein or changing the stop codon to give rise to a longer protein. The dominant ones tended to be missense mutations or in-frame deletions. Among patients with *TPM2* mutations, only one, a patient with NM and pterygia, was homozygous for a recessive mutation. There was one case of mosaicism associated with a heterozygous *TPM2* mutation. In the *TPM3* group, four families showed the recessive mode of inheritance, and there was one case of probable mosaicism. Thus, mosaicism needs to be taken into account in genetic counseling for disorders caused by mutations in either gene.

Type 1 fiber predominance and hypotrophy were common features for all disease entities except for the case of Escobar syndrome. Fiber size disproportion appeared to be consistent among all patients with *TPM3* mutations. Type 1 hypotrophy is to be expected in patients with *TPM3* mutations because of the exclusive expression of Tm3 in type 1 fibers [Perry, 2001], but small type 1 fibers were common also in the group with *TPM2* mutations, despite the expression of Tm2 in both fiber types.

Cap structures and nemaline bodies appeared to be more frequent in biopsies taken at older ages (in three families). This may be explained by the disease process leading to accumulation of sarcomeric protein inclusions over time. Most of the biopsies in CFTD cases had been taken at very young ages (5 months, 6 months, 8 months, 1 year, 14 months, 16 months, 2 years, 11 years, and 29 years). This raises the question of whether the diagnosis of these patients would change if additional biopsies were taken later in life. Such situations have been encountered in a number of CM cases, for example, in one patient with CFTD and cap myopathy caused by a mutation in *TPM2*. In the biopsy taken at the age of 11 years, only fiber type disproportion was noted, whereas in the later biopsy taken at the age of 33 years, cap structures were obvious [Lehtokari et al., 2007]. Caps were more commonly observed in patients with *TPM2* mutations than in patients with *TPM3* mutations (10/41 vs. 5/35; Fig. 4), but the small sample size and the possibility of sampling bias at muscle biopsy hinder any firm conclusion that this should be a significant difference. It has been suggested that cap disease might be considered an early stage or variant of NM [Tajsharghi et al., 2007]. The variable observations of caps and/or nemaline bodies in families and even in individual patients over time [Lehtokari et al., 2007; Tajsharghi et al., 2007] support the concept of cap myopathy being a subcategory of NM.

Patients with hypercontractile molecular phenotypes based on structural analysis of the actin-tropomyosin interface [Marston et al., 2013] (marked by # in the Tables 1 and 2, Supp. Tables S1–S4) more often have contractures of the limb joints (18/19) and jaw (6/19) than those who do not have this type of mutation (2/22 and 1/22). Patients with the nonhypercontractile molecular phenotypes much more often (19/22) have axial contractures

(usually scoliosis, but also rigid spine) than the hypercontractile group (7/19). Presentation was early in both groups, so molecular hypercontractility does not, as has been indicated for patients with the Lys7del mutation in previous studies [Davidson et al., 2013; Memo and Marston, 2013; Mokbel et al., 2013] always cause delayed onset of muscle weakness (Supp. Table S1). Only one hypercontractile molecular phenotype was found in *TPM3* in this study, p.Lys169Glu [Memo and Marston, 2013]. Three are known altogether (unpublished data).

Our results indicate that NM caused by mutations in *TPM2* usually has a milder presentation than NM caused by mutations in *TPM3* (*TPM2*: 10 NM cases, one adult onset, *TPM3*: 10 NM cases, four severe) (Table 3). Recessive disease caused by mutations in these genes appears mostly to be severe. There is no other clear correlation between the type of mutation and the clinical phenotype. For *TPM2*, this was also stated by Tajsharghi et al. (2012) in a recent review.

Biological Relevance

Causative Mutations and Actin Association

In the current models [Perry, 2001; Brown et al., 2005; Li et al., 2011; Memo and Marston, 2013], it is proposed that tropomyosin has a sevenfold repeated amino acid sequence motif that corresponds to the seven actin monomers covered by one tropomyosin. Each motif is subdivided into α - and β -zones. In the relaxed “Off” state, tropomyosin forms contacts with actin through positively charged basic residues in the N-terminal part of an α -zone and acidic residues on the C-terminal side of an α -zone. Ca^{2+} regulation is imposed on actin–tropomyosin by the troponin complex that switches actin–tropomyosin between the “On” and “Off” conformational states. Thus, mutations in tropomyosin potentially can cause muscle dysfunction leading to myopathy through a variety of mechanisms, including effects on formation of dimers, end-to-end interactions, actin binding, and the regulatory interaction with troponin.

Molecular Modeling

Molecular modeling is described in detail by Memo and Marston (2013).

Tropomyosin interaction sites with actin in the “Off” state were precisely mapped by Li et al. (2011). Actin p.Asp25 is predicted to interact with all seven tropomyosin actin-binding repeats. A *TPM2* mutation at one of these tropomyosin interaction sites is p.Lys128Glu and similar *TPM3* mutations are p.Arg91Pro/Cys, the hotspot p.Arg168Cys/Gly/His, and p.Arg245Gly/Ile (Fig. 5). Interestingly, gain-of-function mutations that increase contractility are located in the amino acid next to the Tm actin-binding site: p.Lys7del, p.Lys49del, and p.Arg91Gly in *TPM2* and p.Lys169Glu in *TPM3* [Memo and Marston, 2013]. Actin amino acids 147, 326, and 328 interact with two acidic amino acids in each actin-binding repeat of tropomyosin separated by two to three amino acids at the end of an α -zone. Disease-causing mutations are found affecting only the first acidic amino acid and they are both gain-of-function mutations: *TPM2* p.Glu139del and p.Glu181Lys [Li et al., 2011; Ochala et al., 2012; Memo and Marston, 2013].

In the “On” state, tropomyosin shifts in position relative to the actin filament and myosin contacts are formed [Behrmann et al., 2012]. The “On” state tropomyosin–actin contacts are less well defined and tropomyosin may simply be pushed into its position by strong myosin–actin binding. Overall, fewer disease-causing mutations are adjacent to the proposed “On” state tropomyosin–actin contacts: the *TPM2* mutations p.Asp14Val, p.Glu139del, p.Glu218del, and the *TPM3* mutations p.Leu100Met/Val (Tables 1 and 2; Fig. 5; Supp. Table S5). The other main protein that interacts with tropomyosin is TnT. Of the known *TPM2* mutations, p.Tyr261Cys, and in *TPM3*, p.Met9Arg are involved in the interaction with TnT according to Murakami’s structure of *TPM2* [Murakami et al., 2008] (Fig. 6). Head-to-tail polymerization of tropomyosin is required for actin binding, and has a role in actin filament assembly, and for the regulation of actin–myosin contraction [Murakami et al., 2008]. The mutations p.Ala3Gly, p.Lys7del in *TPM2* and p.Ala4Val, p.Met9Arg, and p.Stop285Ser/Asn in *TPM3* are found at the overlapping region involved in head-to-tail polymerization (Fig. 3).

We hypothesize that the charge changes inherent in most hypercontractile mutations would destabilize the “Off” state and favor the equilibrium toward the “On” state, thus accounting for the higher Ca^{2+} sensitivity as was demonstrated for the *ACTA1* p.Lys326Asn mutation [Orzechowski et al., 2013]. This would be the case with the *TPM2* mutations p.Lys7del, p.Lys49del, p.Arg91Gly, p.Glu139del, and p.Glu181Lys, and the *TPM3* mutation p.Lys169Glu. This seems to correlate with hypercontractile or DA phenotypes that are p.Lys7del, p.Arg91Gly, p.Arg133Trp, and p.Glu181Lys. Congenital muscle weakness correlates with loss-of-function at the molecular level. These mutations are not at the interface of the “Off” state but have an opposite charge change to the gain-of-function mutations and are in a location that could stabilize the “Off” state relative to the “On” state, which may account for the loss-of-function. On the other hand, alteration of the tropomyosin–troponin interface could also have this effect. Mutations shown to be associated with decreased Ca^{2+} sensitivity include *TPM2*-Glu41Lys, *TPM2*-Glu117Lys, *TPM3*-Arg168His, and *TPM3*-Arg245Gly.

In this study, we discuss altogether 30 mutations in *TPM2* and 20 mutations in *TPM3*. More than half of them (18/30 for *TPM2* and 14/20 for *TPM3*) are located in α -zones, which contain residues interacting with actin in the “Off” state. In addition, three (p.Asp2Val, p.Ala3Gly, and p.Lys7del) of the *TPM2* and four (p.Ala4Val, p.Met8Arg, p.Stop285Ser, and p.Stop285Asn) of the *TPM3* mutations are located in the tropomyosin overlapping region (Fig. 3). These regions are essential for tropomyosin function such as actin binding and polymerization. The *TPM3*-Arg168His and *TPM2*-null mutations were found to severely reduce the proportion of strong myosin cross-bridges bound to actin filaments at submaximal Ca^{2+} concentrations and thus to depress force production. The *TPM2*-Glu181Lys mutant was found to have enhanced Ca^{2+} sensitivity and force production [Ochala et al., 2012]. Most mutations are in conserved areas of *TPM2* and *TPM3*. Only five mutations (*TPM2*-Asp2Val, *TPM3*-Gln31Stop, *TPM2*-Glu41Lys, *TPM2*-Leu143Pro, and *TPM3*-Thr252Lys) are in sites where sequence differs between the genes. Four of the amino acids varying between tropomyosins (*TPM2*-Asp2Val, *TPM2*-Glu41Lys, *TPM2*-Leu143Pro,

and TPM3-Thr252Lys) are substituted by similar amino acids, for example, acidic by another acidic one.

Identification of Novel Phosphorylation Sites in Tm2

Phosphorylation has been shown to modify tropomyosin function: it enhances head-to-tail interaction of neighboring tropomyosin dimers, increases binding to TnT and slows relaxation of Ca^{2+} -activated force [Heeley et al., 1989; Hayley et al., 2008; Nixon et al., 2013]. To elucidate a potential role of tropomyosin phosphorylation on pathogenesis, mass spectrometry analyses were performed on purified recombinant tropomyosin, both Tm2 and proteins containing known patient mutations (p.Glu41Lys, p.Lys49del, p.Glu117Lys, p.Glu139del, and p.Gln147Pro). We found four known phosphorylation sites (p.Thr53, p.Thr252, p.Thr282, and p.Ser283) that were present in both the wt and the mutated proteins [Dai et al., 2007; Huttlin et al., 2010]. In addition, we identified four novel phosphorylation sites (p.Thr79, p.Thr108, p.Ser158, and p.Ser206) (Supp. Table S7). The previously unknown phosphorylation sites are situated in the mid region of Tm2 around the mutational hotspot in or near the fourth α -zone (Fig. 3). In all cases examined, the phosphorylation patterns were identical between the wt and the mutated proteins, indicating that changes in phosphorylation patterns are not the main cause of disease for the mutations studied. Also, no patient mutations have been found in the phosphorylated residues in Tm2 and Tm3. Interestingly, in Tm3, only the previously known p.Ser283 was found to be phosphorylated, which corresponds to p.Ser283 in Tm2.

Conclusions

In this report, we have compiled, to the best of our knowledge, all previously reported and novel mutations of *TPM2* and *TPM3*. Altogether we analyzed 27 mutations causing an amino acid change and 3 mutations predicted to affect splicing in *TPM2* and 20 mutations in *TPM3*. Patients with *TPM2* mutations tended to present with milder symptoms than those with *TPM3* mutations, DA being present only in the *TPM2* group. Recessive disease was usually more severe than the dominantly inherited forms. Fiber-type disproportion was consistent in the *TPM3* group and was also common with mutations of *TPM2*. Patients with hypercontractile molecular phenotypes more often had contractures of the limb joints and jaw than those with nonhypercontractile molecular phenotypes, whereas those with no hypercontractility more commonly had spinal deformities or rigidity. No difference was found in the age of onset.

We show that most mutations affect actin association (18/30 in *TPM2* and 13/20 in *TPM3*), resulting in the clinical and histological pictures of NM, cap myopathy, core-rod myopathy, CFTD, CM, DA, and Escobar syndrome with myopathy. Six mutations cause increased Ca^{2+} sensitivity resulting in hypercontractile phenotypes. Among the remaining mutations, all five so far tested caused decreased Ca^{2+} sensitivity. Three of the *TPM2* and four of the *TPM3* mutations were located in the tropomyosin overlapping region affecting head-to-tail binding. We report four novel phosphorylation sites in β -tropomyosin. Although phosphorylation is known to be linked to tropomyosin–actin association, we found no

mutations in phosphorylated residues, nor altered phosphorylation patterns in purified tropomyosin proteins containing known patient mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr Homa Tajsharghi for helpful discussion of tropomyosin expression during development and Elizabeth DeChene, MS, CGC, for helpful discussions and assistance with clinical diagnostic testing. We would like to thank Leigh B. Waddell, PhD, and we also thank Fabiana Fattori, PhD, for screening for mutations in *TPM2* and *TPM3* patients in Italy and Adele D'Amico, MD, PhD, for clinical and genetic characterization of congenital myopathies and coordinating the international network of neuromuscular disorders in Italy. F.P. holds a Career Award for Medical Scientists from the Burroughs Wellcome Fund.

Contract grant sponsors: National Institute of Arthritis and Musculoskeletal and Skin Diseases of the NIH (grant R01 AR044345); Muscular Dystrophy Association (MDA201302); Australian National Health, Medical Research Council (NHMRC) Project (grant APP1022707); NHMRC Centre of Research Excellence (grant APP1031893); NHMRC of Australia (APP1035828, APP1026933); Telethon GUP08005; European Union Seventh Framework Programme (FP7/2007–2013) (grant agreement no. 305444 (RD-Connect) and 305121 (Neuromics)); Association Française contre les Myopathies; Sigrid Jusélius Foundation; Academy of Finland; Finska Läkaresällskapet; Medicinska un-derstödsföreningen Liv och Hälsa r.f.; DHFMR; Foundation for Building Strength for Nemaline Myopathy; Ricerca Finalizzata Ministry of Health; British Heart Foundation.

References

- Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010; 7:248–249. [PubMed: 20354512]
- Akkari PA, Song Y, Hitchcock-DeGregori S, Blechynden L, Laing N. Expression and biological activity of baculovirus generated wild-type human slow alpha tropomyosin and the Met9Arg mutant responsible for a dominant form of nemaline myopathy. *Biochem Biophys Res Commun*. 2002; 296:300–304. [PubMed: 12163017]
- Barua B, Pamula MC, Hitchcock-DeGregori SE. Evolutionarily conserved surface residues constitute actin binding sites of tropomyosin. *Proc Natl Acad Sci USA*. 2011; 108:10150–10155. [PubMed: 21642532]
- Behrmann E, Muller M, Penczek PA, Mannherz HG, Manstein DJ, Raunser S. Structure of the rigor actin–tropomyosin–myosin complex. *Cell*. 2012; 150:327–338. [PubMed: 22817895]
- Brooke, MH. *Clinical studies in myology*. Amsterdam: Excerpta Medica; 1973. p. 147
- Brown JH, Zhou Z, Reshetnikova L, Robinson H, Yammani RD, Tobacman LS, Cohen C. Structure of the mid-region of tropomyosin: bending and binding sites for actin. *Proc Natl Acad Sci USA*. 2005; 102:18878–18883. [PubMed: 16365313]
- Clarke NF, Domazetovska A, Waddell L, Kornberg A, McLean C, North KN. Cap disease due to mutation of the beta-tropomyosin gene (TPM2). *Neuromuscul Disord*. 2009; 19:348–351. [PubMed: 19345583]
- Clarke NF, Kolski H, Dye DE, Lim E, Smith RL, Patel R, Fahey MC, Bellance R, Romero NB, Johnson ES, Labarre-Vila A, Monnier N, Laing NG, North KN. Mutations in TPM3 are a common cause of congenital fiber type disproportion. *Ann Neurol*. 2008; 63:329–337. [PubMed: 18300303]
- Conen PE, Murphy EG, Donohue WL. Light and electron microscopic studies of “myogranules” in a child with hypotonia and muscle weakness. *Can Med Assoc J*. 1963; 89:983–986. [PubMed: 14076166]
- Cooley BC, Bergtrom G. Multiple combinations of alternatively spliced exons in rat tropomyosin-alpha gene mRNA: evidence for 20 new isoforms in adult tissues and cultured cells. *Arch Biochem Biophys*. 2001; 390:71–77. [PubMed: 11368517]

- Cooper JA. Actin dynamics: tropomyosin provides stability. *Curr Biol*. 2002; 12:R523–R525. [PubMed: 12176375]
- Daentl DL, Berg BO, Layzer RB, Epstein CJ. A new familial arthrogryposis without weakness. *Neurology*. 1974; 24:55–60. [PubMed: 4855665]
- Dai J, Jin WH, Sheng QH, Shieh CH, Wu JR, Zeng R. Protein phosphorylation and expression profiling by yin-yang multidimensional liquid chromatography (yin-yang MDLC) mass spectrometry. *J Proteome Res*. 2007; 6:250–262. [PubMed: 17203969]
- Davidson AE, Siddiqui FM, Lopez MA, Lunt P, Carlson HA, Moore BE, Love S, Born DE, Roper H, Majumdar A, Jayadev S, Underhill HR, et al. Novel deletion of lysine 7 expands the clinical, histopathological and genetic spectrum of TPM2-related myopathies. *Brain*. 2013; 136:508–521. [PubMed: 23413262]
- De Paula AM, Franques J, Fernandez C, Monnier N, Lunardi J, Pellissier JF, Figarella-Branger D, Pouget J. A TPM3 mutation causing cap myopathy. *Neuromuscul Disord*. 2009; 19:685–688. [PubMed: 19553118]
- den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat*. 2000; 15:7–12. [PubMed: 10612815]
- Dufour C, Weinberger RP, Schevzov G, Jeffrey PL, Gunning P. Splicing of two internal and four carboxyl-terminal alternative exons in nonmuscle tropomyosin 5 pre-mRNA is independently regulated during development. *J Biol Chem*. 1998; 273:18547–18555. [PubMed: 9660825]
- Durling HJ, Reilich P, Muller-Hocker J, Mendel B, Pongratz D, Wallgren-Pettersson C, Gunning P, Lochmuller H, Laing NG. De novo missense mutation in a constitutively expressed exon of the slow alpha-tropomyosin gene TPM3 associated with an atypical, sporadic case of nemaline myopathy. *Neuromuscul Disord*. 2002; 12:947–951. [PubMed: 12467750]
- Fidzianska A, Badurska B, Ryniewicz B, Dembek I. “Cap disease”: new congenital myopathy. *Neurology*. 1981; 31:1113–1120. [PubMed: 7196531]
- Gordon AM, Homsher E, Regnier M. Regulation of contraction in striated muscle. *Physiol Rev*. 2000; 80:853–924. [PubMed: 10747208]
- Gunning PW, Schevzov G, Kee AJ, Hardeman EC. Tropomyosin isoforms: divining rods for actin cytoskeleton function. *Trends Cell Biol*. 2005; 15:333–341. [PubMed: 15953552]
- Hayley M, Chevaldina T, Mudalige WA, Jackman DM, Dobbin AD, Heeley DH. Shark skeletal muscle tropomyosin is a phosphoprotein. *J Muscle Res Cell Motil*. 2008; 29:101–107. [PubMed: 18763042]
- Heeley DH, Watson MH, Mak AS, Dubord P, Smillie LB. Effect of phosphorylation on the interaction and functional properties of rabbit striated muscle alpha alpha-tropomyosin. *J Biol Chem*. 1989; 264:2424–2430. [PubMed: 2521628]
- Hitchcock-DeGregori SE, Song Y, Moraczewska J. Importance of internal regions and the overall length of tropomyosin for actin binding and regulatory function. *Biochemistry*. 2001; 40:2104–2112. [PubMed: 11329279]
- Holmes KC, Popp D, Gebhard W, Kabsch W. Atomic model of the actin filament. *Nature*. 1990; 347:44–49. [PubMed: 2395461]
- Huttlin EL, Jedrychowski MP, Elias JE, Goswami T, Rad R, Beausoleil SA, Villen J, Haas W, Sowa ME, Gygi SP. A tissue-specific atlas of mouse protein phosphorylation and expression. *Cell*. 2010; 143:1174–1189. [PubMed: 21183079]
- Klein A1, Lillis S, Munteanu I, Scoto M, Zhou H, Quinlivan R, Straub V, Manzur AY, Roper H, Jeannot PY, Rakowicz W, Jones DH, Jensen UB, Wraige E, Trump N, Schara U, Lochmuller H, Sarkozy A, Kingston H, Norwood F, Damian M, Kirschner J, Longman C, Roberts M, Auer-Grumbach M, Hughes I, Bushby K, Sewry C, Robb S, Abbs S, Jungbluth H, Muntoni F. Clinical and genetic findings in a large cohort of patients with ryanodine receptor 1 gene-associated myopathies. *Hum Mutat*. 2012; 33:981–988. [PubMed: 22473935]
- Krakowiak PA, O’Quinn JR, Bohnsack JF, Watkins WS, Carey JC, Jorde LB, Bamshad M. A variant of Freeman-Sheldon syndrome maps to 11p15.5-pter. *Am J Hum Genet*. 1997; 60:426–432. [PubMed: 9012416]

- Lawlor MW, Dechene ET, Roumm E, Geggel AS, Moghadaszadeh B, Beggs AH. Mutations of tropomyosin 3 (TPM3) are common and associated with type 1 myofiber hypotrophy in congenital fiber type disproportion. *Hum Mutat.* 2010; 31:176. [PubMed: 19953533]
- Lehtokari VL, Ceuterick-de Groote C, de Jonghe P, Marttila M, Laing NG, Pelin K, Wallgren-Pettersson C. Cap disease caused by heterozygous deletion of the beta-tropomyosin gene TPM2. *Neuromuscul Disord.* 2007; 17:433–442. [PubMed: 17434307]
- Li XE, Tobacman LS, Mun JY, Craig R, Fischer S, Lehman W. Tropomyosin position on F-actin revealed by EM reconstruction and computational chemistry. *Biophys J.* 2011; 100:1005–1013. [PubMed: 21320445]
- Lin JJ, Warren KS, Wamboldt DD, Wang T, Lin JL. Tropomyosin isoforms in nonmuscle cells. *Int Rev Cytol.* 1997; 170:1–38. [PubMed: 9002235]
- Mak A, Smillie LB, Barany M. Specific phosphorylation at serine-283 of alpha tropomyosin from frog skeletal and rabbit skeletal and cardiac muscle. *Proc Natl Acad Sci USA.* 1978; 75:3588–3592. [PubMed: 278975]
- Marston S, Memo M, Messer A, Papadaki M, Nowak K, McNamara E, Ong R, El-Mezgueldi , Li X, Lehman W. Mutations in repeating structural motifs of tropomyosin cause gain of function in skeletal muscle myopathy patients. *Hum Mol Genet.* 2013; 22:4978–4987. [PubMed: 23886664]
- Marttila M, Lemola E, Wallefeld W, Memo M, Donner K, Laing NG, Marston S, Gronholm M, Wallgren-Pettersson C. Abnormal actin binding of aberrant beta-tropomyosins is a molecular cause of muscle weakness in TPM2-related nemaline and cap myopathy. *Biochem J.* 2012; 442:231–239. [PubMed: 22084935]
- Matsumura F, Yamashiro-Matsumura S, Lin JJ. Isolation and characterization of tropomyosin-containing microfilaments from cultured cells. *J Biol Chem.* 1983; 258:6636–6644. [PubMed: 6133866]
- McLachlan AD, Stewart M. The 14-fold periodicity in alpha-tropomyosin and the interaction with actin. *J Mol Biol.* 1976; 103:271–298. [PubMed: 950663]
- Memo M, Marston S. New structures of the actin-tropomyosin interface can explain the functional consequences of mutations in skeletal muscle tropomyosin that cause congenital myopathies. *J Muscle Res Cell Motil.* 2013; 34:165–169. [PubMed: 23719967]
- Mokbel N, Ilkovski B, Kreissl M, Memo M, Jeffries CM, Marttila M, Lehtokari VL, Lemola E, Gronholm M, Yang N, Menard D, Marcotelles P, et al. K7del is a common TPM2 gene mutation associated with nemaline myopathy and raised myofibre calcium sensitivity. *Brain.* 2013; 136:494–507. [PubMed: 23378224]
- Monnier N, Lunardi J, Marty I, Mezin P, Labarre-Vila A, Dieterich K, Jouk PS. Absence of beta-tropomyosin is a new cause of Escobar syndrome associated with nemaline myopathy. *Neuromuscul Disord.* 2009; 19:118–123. [PubMed: 19155175]
- Munot P, Lashley D, Jungbluth H, Feng L, Pitt M, Robb SA, Palace J, Jayawant S, Kennet R, Beeson D, Cullup T, Abbs S, Laing N, Sewry C, Muntoni F. Congenital fibre type disproportion associated with mutations in the tropomyosin 3 (TPM3) gene mimicking congenital myasthenia. 2010; 20:796–800.
- Murakami K, Stewart M, Nozawa K, Tomii K, Kudou N, Igarashi N, Shirakihara Y, Wakatsuki S, Yasunaga T, Wakabayashi T. Structural basis for tropomyosin overlap in thin (actin) filaments and the generation of a molecular swivel by troponin-T. *Proc Natl Acad Sci USA.* 2008; 105:7200–7205. [PubMed: 18483193]
- Muthuchamy M, Pajak L, Howles P, Doetschman T, Wieczorek DF. Developmental analysis of tropomyosin gene expression in embryonic stem cells and mouse embryos. *Mol Cell Biol.* 1993; 13:3311–3323. [PubMed: 7684495]
- Nixon BR, Liu B, Scellini B, Tesi C, Piroddi N, Ogut O, John Solaro R, Ziolo MT, Janssen PM, Davis JP, Poggesi C, Biesiadecki BJ. Tropomyosin ser-283 pseudo-phosphorylation slows myofibril relaxation. *Arch Biochem Biophys.* 2013; 535:30–38. [PubMed: 23232082]
- Norum RA, James VL, Mabry CC. Pterygium syndrome in three children in a recessive pedigree pattern. *Birth Defects Orig Art Ser.* 1969; 2:233–235.

- Ochala J, Gokhin DS, Penisson-Besnier I, Quijano-Roy S, Monnier N, Lunardi J, Romero NB, Fowler VM. Congenital myopathy-causing tropomyosin mutations induce thin filament dysfunction via distinct physiological mechanisms. *Hum Mol Genet.* 2012; 21:4473–4485. [PubMed: 22798622]
- O'Connell KL, Stults JT. Identification of mouse liver proteins on two-dimensional electrophoresis gels by matrix-assisted laser desorption/ionization mass spectrometry of in situ enzymatic digests. *Electrophoresis.* 1997; 18:349–359. [PubMed: 9150913]
- Ohlsson M, Quijano-Roy S, Darin N, Brochier G, Lacene E, Avila-Smirnow D, Fardeau M, Oldfors A, Tajsharghi H. New morphologic and genetic findings in cap disease associated with beta-tropomyosin (TPM2) mutations. *Neurology.* 2008; 71:1896–1901. [PubMed: 19047562]
- Ohman T, Lietzén N, Välimäki E, Meljchorsen J, Matikainen S, Nyman TA. Cytosolic RNA recognition pathway activates 14-3-3 protein mediated signaling and caspase-dependent disruption of cytoskeleton network in human keratinocytes. *J Proteome Res.* 2010; 9:1549–1564. [PubMed: 20070120]
- Orzechowski M, Fischer S, Lehman W. Influence of actin mutation on the energy landscape of actin-tropomyosin filaments. *Biophys J Biophys Soc.* 2013; 104(S1):480a.
- Penisson-Besnier I, Monnier N, Toutain A, Dubas F, Laing N. A second pedigree with autosomal dominant nemaline myopathy caused by TPM3 mutation: a clinical and pathological study. *Neuromuscul Disord.* 2007; 17:330–337. [PubMed: 17376686]
- Perry SV. Vertebrate tropomyosin: distribution, properties and function. *J Muscle Res Cell Motil.* 2001; 22:5–49. [PubMed: 11563548]
- Phillips GN Jr, Lattman EE, Cummins P, Lee KY, Cohen C. Crystal structure and molecular interactions of tropomyosin. *Nature.* 1979; 278:413–417. [PubMed: 450047]
- Pittenger MF, Kazzaz JA, Helfman DM. Functional properties of non-muscle tropomyosin isoforms. *Curr Opin Cell Biol.* 1994; 6:96–104. [PubMed: 8167032]
- Ribolow H, Barany M. Phosphorylation of tropomyosin in live frog muscle. *Arch Biochem Biophys.* 1977; 179:718–720. [PubMed: 851366]
- Shihab HA, Gough J, Cooper DN, Stenson PD, Barker GL, Edwards KJ, Day IN, Gaunt TR. Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models. *Hum Mutat.* 2013; 34:57–65. [PubMed: 23033316]
- Shy GM, Engel WK, Somers JE, Wanko T. Nemaline myopathy: a new congenital myopathy. *Brain.* 1963; 86:793–810. [PubMed: 14090530]
- Tajsharghi H, Ohlsson M, Lindberg C, Oldfors A. Congenital myopathy with nemaline rods and cap structures caused by a mutation in the beta-tropomyosin gene (TPM2). *Arch Neurol.* 2007; 64:1334–1338. [PubMed: 17846275]
- Tajsharghi H, Ohlsson M, Palm L, Oldfors A. Myopathies associated with beta-tropomyosin mutations. *Neuromuscul Disord.* 2012; 22:923–933. [PubMed: 22749895]
- Tasca G, Fattori F, Ricci E, Monforte M, Rizzo V, Mercuri E, Bertini E, Silvestri G. Somatic mosaicism in TPM2-related myopathy with nemaline rods and cap structures. *Acta Neuropathol.* 2013; 125:169–171. [PubMed: 23015096]
- Wallgren-Pettersson C, Pelin K, Nowak KJ, Muntoni F, Romero NB, Goebel HH, North KN, Beggs AH, Laing NG. ENMCInternational Consortium On Nemaline Myopathy. Genotype-phenotype correlations in nemaline myopathy caused by mutations in the genes for nebulin and skeletal muscle alpha-actin. *Neuromuscul Disord.* 2004; 14:461–470. [PubMed: 15336686]
- Wallgren-Pettersson C, Sewry CA, Nowak KJ, Laing NG. Nemaline myopathies. *Semin Pediatr Neurol.* 2011; 18:230–238. [PubMed: 22172418]

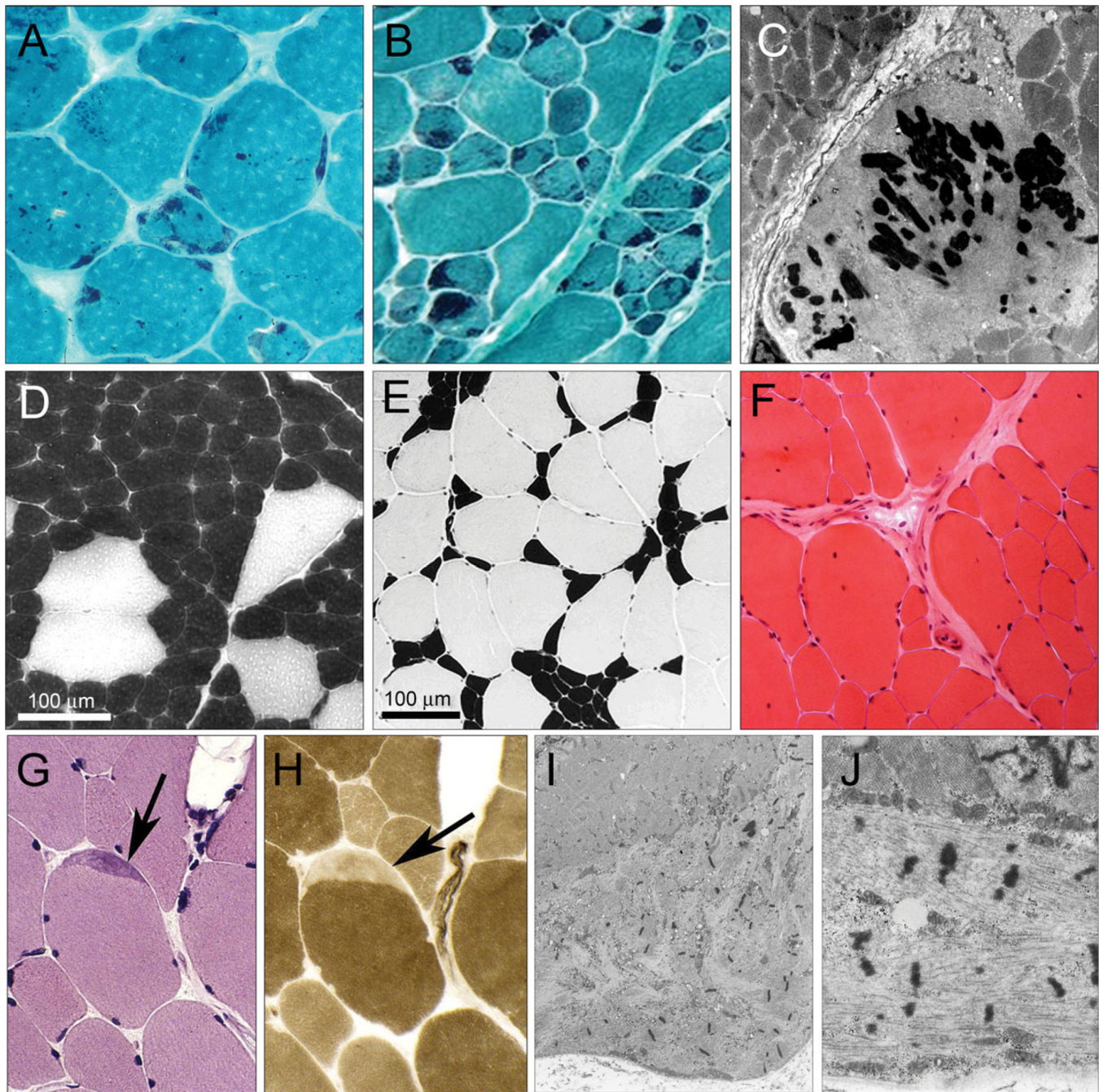


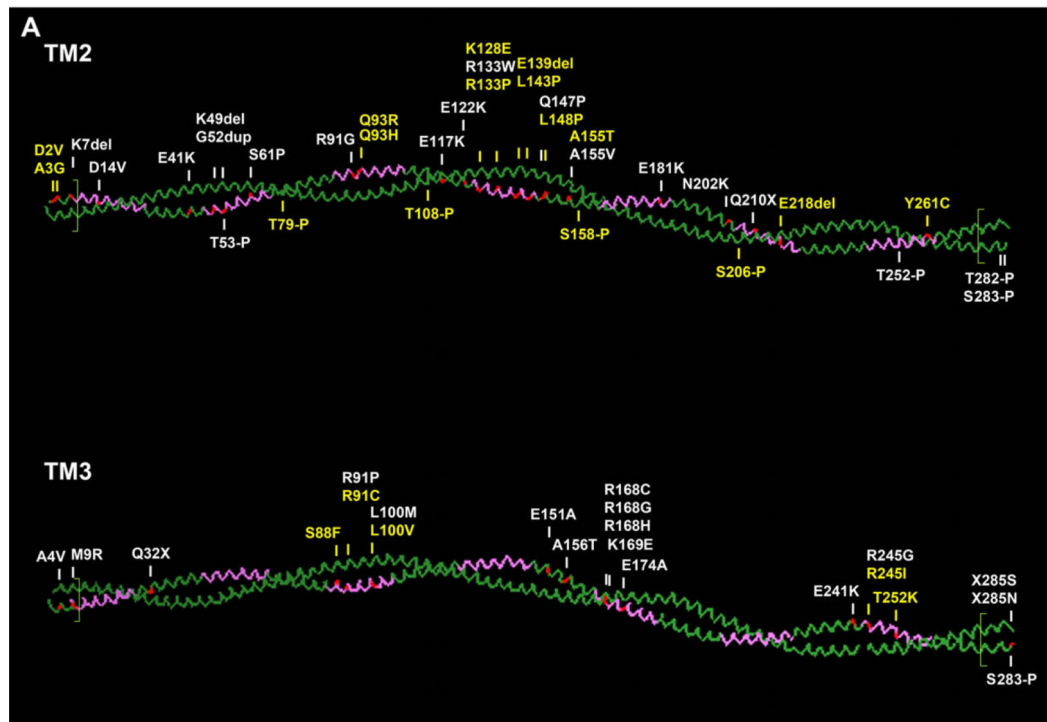
Figure 1.

Histological characteristics of NM, cap myopathy, and CFTD. Histology images showing the range of abnormalities in skeletal muscle that can arise with mutations in tropomyosin. **A–C:** NM. In *TPM2*, NM (**A**: p.K7del mutation) rods are often randomly scattered. In *TPM3*, NM (**B**: p.M9R), rods are confined to type 1 fibers that are usually hypotrophic. **C:** Nemaline bodies on electron microscopy. **D–F:** CFTD due to mutations in *TPM3D* and **E:** ATPase (4.3) showing the consistent difference in size between type 1 fibers (dark) and type 2 fibers (pale). Type 1 fiber predominance is common (**D**) but a range is seen (**E**). **D:**

Increased internalized nuclei in large (type 2) fibers in an adult CFTD patient with the *TPM3* p.L100M mutation. **G–J**: Cap myopathy due to the *TPM2* p.Glu139del mutation. Caps are most reliably seen on ATPase stains (**H**) but are sometimes visible on H&E stains (**G**) or oxidative stains. **I** and **J**: Electron microscopy of a typical cap structure.



Figure 2.
Distal arthrogryposis patient. Little girl with distal arthrogryposis and suspected myopathy.



B

CLUSTAL 2.0.5 multiple sequence alignment

[illegible]

Figure 3.

Sequence comparison of Tm1, Tm2, and Tm3 and Tm2–Tm3 dimer with disease-causing mutations and phosphorylation sites. **A:** Schematic presentation of Tm2 and Tm3 dimers with disease-causing mutations (shown in red and above the molecules), α -zones (purple), and overlapping regions (separated by green lines in N- and C-terminal ends of the molecules). Phosphorylation sites are marked underneath the molecules. Novel mutations/phosphorylation sites are shown in yellow. The figure was created using the PyMol software (<http://www.pymol.org>) and the Protein Databank structure 1C1GA. **B:** Sequence

comparison of Tm1, Tm2, and Tm3. Mutations are indicated in the sequence by red dots. In the Tm3 protein amino acid sequence (P06753), the initiation codons have been processed, thus the nomenclature is different compared with that in other figures. Stars underneath the sequences indicate conserved amino acids, and dots show the sites where sequences diverge.

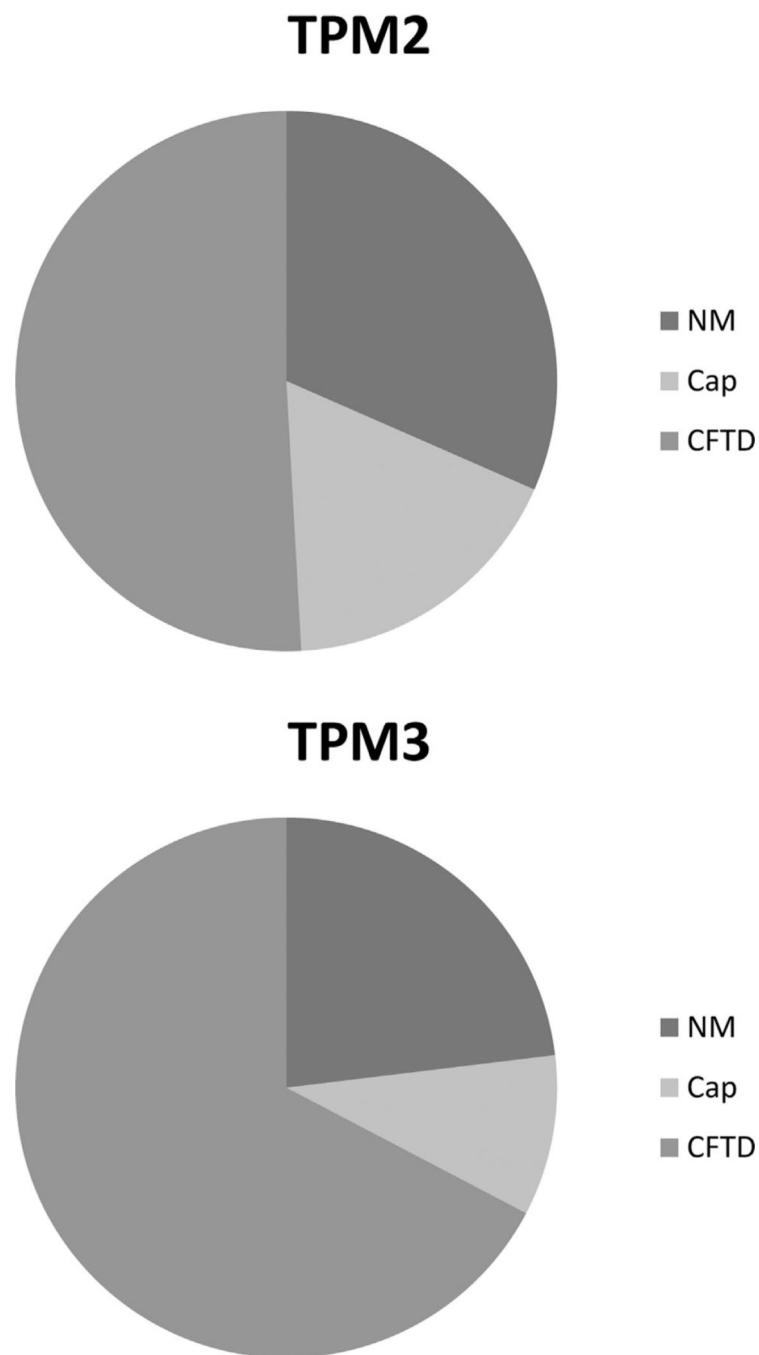
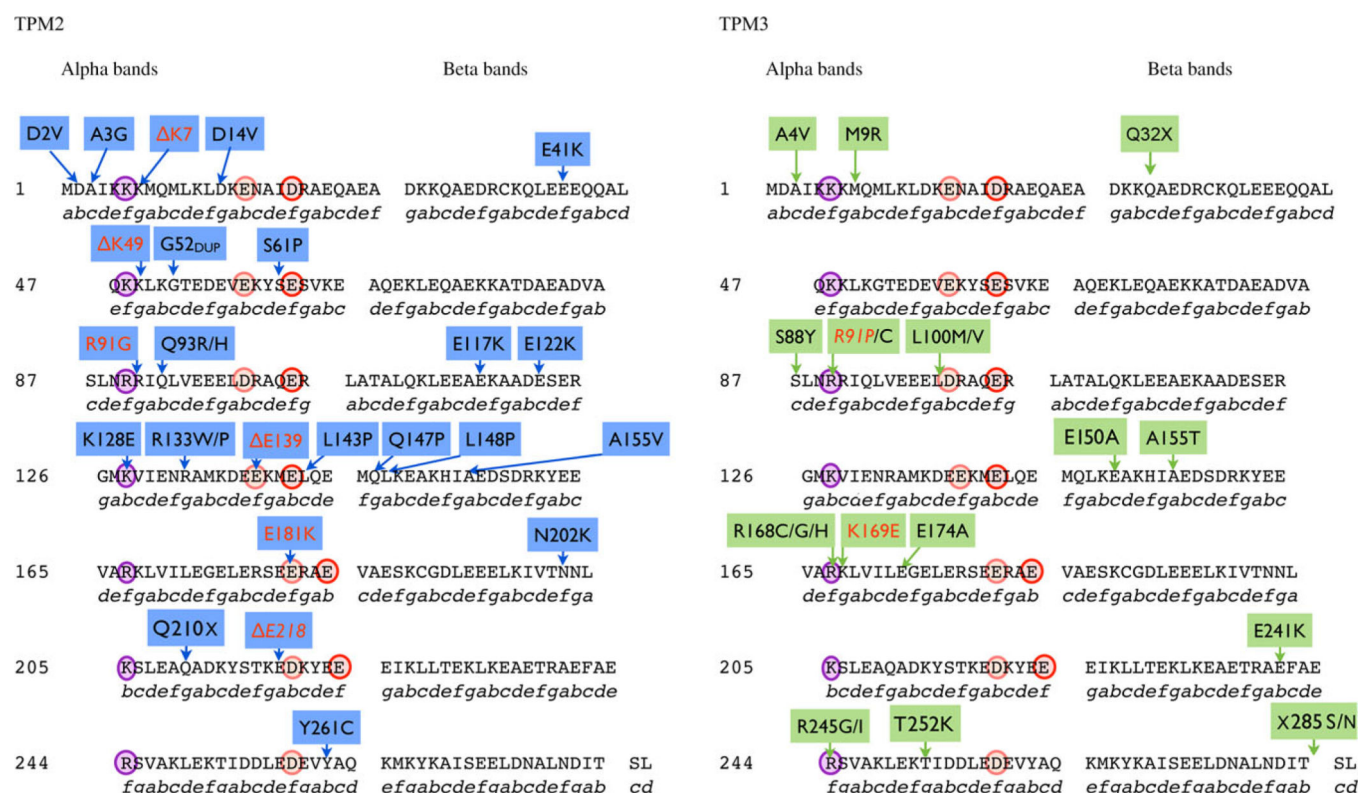


Figure 4.

A pie chart showing histopathological characteristics in biopsies from patients with mutations in the *TPM2* and *TPM3* genes. Abbreviations: NM, nemaline bodies; Cap, Cap structures; and CFTD, fiber size disproportion.

**Figure 5.**

On-state actin–tropomyosin contacts and disease mutations. The Tm2 (β-Tm) sequence and the Tm3 (γ-Tm) sequence divided into seven quasi-repeats and α- and β-bands as defined by Mclachlan and Stewart (1976). The purple circles highlight residues interacting with actin Asp25; the orange circles highlight the residues interacting with actin R147, K326, and K328 as defined by Li et al. (2010). The Tm2 mutations are indicated in blue boxes; the Tm3 mutations are indicated in green boxes. The mutations increasing Ca^{2+} -sensitivity are written in red, whereas those decreasing it are in black.

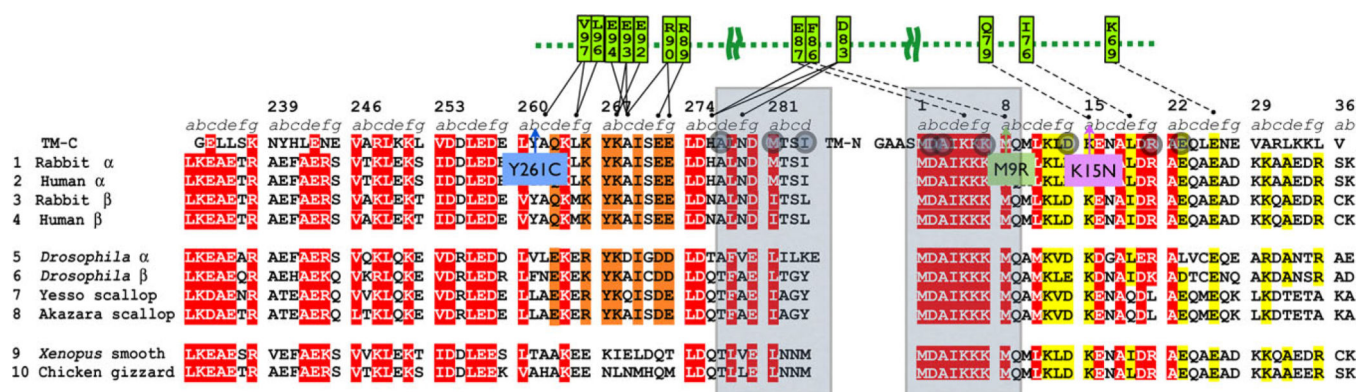


Figure 6.

Tropomyosin end-end overlap and interactions of tropomyosin with TnT. Tropomyosin end-end overlap (one to nine and 277–284, gray boxes) and interactions of tropomyosin with TnT based on the model of Murakami et al. (2008). Mutations in overlap zone are shown as gray circles; pink outline *TPM1* (cardiomyopathy), blue outline *TPM2*. Only a few mutations are involved in contacts with TnT (green) in this region of tropomyosin. *TPM2* Y261C is next to A262, M9R is supposed to bind to F86 in TnT, and K15N (*TPM1*) is supposed to bind with TnT Q79.

Table 1

Novel and Recurrent *TPM2* Mutations in 28 Patients from 27 Families

Family	Patient ID, gender	Mutation(s) in cDNA RefSeq NM_003289.3	Altered protein site and predicted effect	Diagnosis	Phenotype	PolyPhen-2/FATHMM prediction	Reference
<i>TPM2</i> novel mutations							
1	2,693, M	c.8C>G	p.Ala3Gly	NM	Proximal muscle weakness (66 years)	Benign/damaging	Present study (Family 1)
2#	20-625, F	c.41A>T	p.Asp14Val	NM	progressive course of the disease	Possibly damaging/damaging	Present study (Family 12)
3	20-561, F	c.124G>A	p.Glu41Lys	NM	Severe neonatal hypotonia, weakness of neck flexors	Benign/tolerated	Present study (Family 15)
4	F	c.240+2T>G	Spl	CFTD	Distal arthrogryposis and weakness	-	Present study (Family 19)
5	F	c.240+5G>A	Spl	DA	Hypotonia, joint contractures, and bilateral club foot	-	Present study (Family 20)
6	20-601, F	c.279G>C	p.Gln93His	CM	Neonatal hypotonia with respiratory difficulties	Probably damaging/damaging	Present study (Family 22)
7#	F	c.349G>A	p.Glu117Lys	DA	Hypotonia at birth, failure to thrive, scoliosis	Possibly damaging/damaging	Present study (Family 24)
8#	20-393, M	c.382A>G	p.Lys128Glu	CM	Hypotonia at birth mild weakness	Probably damaging/damaging	Present study (Family 26)
9#	3,603, F	c.397C>T	p.Arg133Trp	CFTD	Progressive weakness	Possibly damaging/damaging	Present study (Family 28)
10#	M	c.398G>C	p.Arg133Pro	CFTD	Severe hypotonia and respiratory failure (at birth)	Probably damaging/damaging	Present study (Family 29)
11	3,143, F	c.428T>C	p.Leu143Pro	NM CFTD	Distal weakness of lower limbs	Possibly damaging/damaging	Present study (Family 35)
12#	3,653, F	c.443T>C	p.Leu148Pro	CFTD	Presented with muscle weakness subsequent to infection	Possibly damaging/damaging	Present study (Family 37)
Recurrent mutations							
13#	Family A, F	c.19 21delAAG	p.Lys7del	NM	Contractures, mild proximal leg weakness	-	Mokbel et al. (2013) (Family 2)
14#	Family B, M	c.19 21delAAG	p.Lys7del	NM	Contractures, marked fatigue	-	Mokbel et al. (2013) (Family 3)
15#	Family C, F	c.19 21delAAG	p.Lys7del	NM	Contractures, proximal limb weakness	-	Mokbel et al. (2013) (Family 4)
16#	Family D, M	c.19 21delAAG	p.Lys7del	NM	Contractures, mild proximal limb and distal leg weakness	-	Mokbel et al. (2013) (Family 5)

Family	Patient ID, gender	Mutation(s) in cDNA RefSeq NM_003289.3	Altered protein site and predicted effect	Diagnosis	Phenotype	PolyPhen-2/FATHMM prediction	Reference
17#	Family E, 1,653, F	c.19 21delAAG	p.Lys7del	NM	Quadriceps weakness, ambulation lost (62 years)	–	Mokbel et al. (2013) (Family 6)
18#	20-747, F	c.19 21delAAG	p.Lys7del	NM	Joint contractures, no weakness	–	Present study (Family 7)
19#	Family 1 M	c.19 21delAAG	p.Lys7del	CRM	Contractures, gait unsteadiness	–	Davidson et al. (2012) (Family 8)
20#	Family 2, F	c.19 21delAAG	p.Lys7del	DA type 7	Limited ambulation, congenital hip dislocations	–	Davidson et al. (2012) (Family 9)
21#	Family 3, F	c.19 21delAAG	p.Lys7del	DA type 7	Contractures, difficulties running, gait difficulties	–	Davidson et al. (2012) (Family 10)
22#	Family 4, F	c.19 21delAAG	p.Lys7del	DA type 7	Walked with straightened legs and had “stiff posture”	–	Davidson et al. (2012) (Family 11)
23#	291-A, B F, M	c.415 417delGAG	p.Glu139del	NM Cap	Slowly progressive muscle weakness, rigid spine	–	Tasca et al. (2013) (Family 30)
24#	F	c.415 417delGAG	p.Glu139del	CFTD	Able to walk, cervical scoliosis	–	Present study (Family 31)
25#	3,073, M	c.415 417delGAG	p.Glu139del	Cap CFTD	Generalized muscle weakness, never able to run	–	Lehtokari et al. (2007) (Family 32)
26#	F	c.415 417delGAG	p.Glu139del	Cap	Generalized muscle wasting, running in childhood	–	Clarke et al. (2009) (Family 33)
27#	F	c.415 417delGAG	p.Glu139del	Cap CFTD	Proximal muscles weaker than distal	–	Present study (Family 34)

PolyPhen-2 and FATHMM predictions indicate the possible pathogenicity of the missense mutations.

The reference column links families to the corresponding Supp. Tables S1 and S2. The possible pathogenicity of the mutations was assessed with PolyPhen-2, version 2.2.2, HumVar option, and FATHMM version 2.3 with the prediction algorithm weighted for human mutations and disease ontology as the option for phenotypic associations. Nucleotide numbering according to the *TPM2* cDNA sequence with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_003289.3. Amino acid coordinates are provided relative to NP_003280.2; the ATG translation initiation codon is codon 1.

NM, nemaline myopathy; Cap, cap myopathy; CFTD, congenital fiber-type disproportion; DA, distal arthrogryposis; CRM, core-rod myopathy; CM, undefined congenital myopathy; spl, splice-site mutation; #, gain-of-function mutation resulting in hypercontractile phenotypes; M, male; F, female.

Table 2

Novel and Recurrent *TPM3* Mutations in 24 Patients from 22 Families

Family	Patient ID, gender	Mutation(s) in cDNA RefSeq NM_152263.3	Altered protein site and predicted effect	Diagnosis	Phenotype	PolyPhen-2/FA THMM prediction	Reference
<i>TPM3</i> novel mutations							
1	3,363, M	c.263C>T	(p.Ser87Phe) p.Ser88Phe	NM Cap	Myalgia, progressive weakness	Probably damaging/damaging	Present study (Family 3)
2	20-799, M	c.271C>T	(p.Arg90Cys) p.Arg91Cys	CM	Moderate scapular and pelvic weakness	Probably damaging/damaging	Present study (Family 5)
3	F	c.298C>G	(p.Leu99Val) p.Leu100Val	CFTD	Neonatal hypotonia, head lag, running	Benign/damaging	Present study (Family 7)
4	20-607, F	c.452A>C	(p.Glu150Ala) p.Glu151Ala	Cap	Weakness of neck flexor	Probably damaging/damaging	Present study (Family 8)
5	20-834, F	c.734G>T	(p.Arg244Ile) p.Arg245Ile	Cap	Proximal weakness, able to sit but not to stand up	Probably damaging/damaging	Present study (Family 29)
6	20-872, F	c.758C>A	(p.Thr252Lys) p.Thr253Lys	CM	Myopathic facies, walking	Possibly damaging/tolerated	Present study (Family 30)
Recurrent mutations							
7	F	c.502C>T	(p.Arg167Cys) p.Arg168Cys	NM	Proximal and distal weakness, walking with difficulty	Probably damaging/damaging	Present study (Family 10)
8	1,963, F	c.502C>T	(p.Arg167Cys) p.Arg168Cys	CFTD	Moderate weakness, running in childhood, walks, stairs difficult	Probably damaging/damaging	Clarke et al. (2008) Family 5 (Family 1)
9	BOS1109-1, F	c.502C>T	(p.Arg167Cys) p.Arg168Cys	CFTD	Moderate proximal and distal weakness	Probably damaging/damaging	Lawlor et al. (2010) (Family 12)
10	20-837, M	c.502C>T	(p.Arg167Cys) p.Arg168Cys	NM	Walks with support, kyphosis	Probably damaging/damaging	Present study (Family 13)
11	M	c.502C>T	(p.Arg167Cys) p.Arg168Cys	CFTD	Weakness of the feet, ambulant, slow runner	Probably damaging/damaging	Present study (Family 14)
12	F	c.502C>G	(p.Arg167Gly) p.Arg168Gly	CFTD	Mild facial and neck flexor weakness, slow runner	Probably damaging/damaging	Clarke et al. (2008) (Family 3) (Family 15)
13	F	c.503G>A	(p.Arg167His) p.Arg168His	CFTD	Neck flexor weakness, achieved walking	Benign/damaging	Present study (Family 16)
14	M	c.503G>A	(p.Arg167His) p.Arg168His	CFTD	Hypotonic, mild weakness in distal muscles, climbs stairs	Benign/damaging	Present study (Family 17)
15	F, M	c.503G>A	(p.Arg167His)	NM	Proximal limb girdle weakness, mild facial weakness, runs	Benign/damaging	Durling et al. (2002)
			p.Arg168His	CFTD			Clarke et al. (2008)

Family	Patient ID, gender	Mutation(s) in cDNA RefSeq NM_152263.3	Altered protein site and predicted effect	Diagnosis	Phenotype	PolyPhen-2/FATHMM prediction	Reference
16	M	c.503G>A	(p.Arg167His) p.Arg168His	NM	Walks, has never been able to run or jump	Benign/damaging	(Family 6) (Family 18) Penisson-Besnier et al. (2007) (Family 19)
17	M	c.503G>A	(p.Arg167His) p.Arg168His	Cap	Distal weakness of lower limbs, unable to run	Benign/damaging	De Paula et al. (2009) (Family 20)
18	BOS 247-4, F	c.503G>A	(p.Arg167His) p.Arg168His	CFTD	Axial weakness, able to jump, but not very high	Benign/damaging	Lawlor et al. (2010) (Family 21)
19	BOS 343-1, M	c.503G>A	(p.Arg167His) p.Arg168His	NM	Difficulty raising arms and holding head, respiration difficulty	Benign/damaging	Lawlor et al. (2010) (Family 22)
20	F	c.503G>A	(p.Arg167His) p.Arg168His (RYR p.Arg3539His)	CFTD	Never able to walk far, run or jump, ambulant	Benign/damaging	Klein et al. (2012) (Family 23)
21	F	c.503G>A	(p.Arg167His) p.Arg168His	CFTD	Spinal rigidity and scoliosis, facial weakness	Benign/damaging	Munot et al. (2010) (Family 24)

PolyPhen-2 and FATHMM predictions indicate the possible pathogenicity of the missense mutations. The reference column links families to the corresponding Supp. Tables S3 and S4. The possible pathogenicity of the mutations was assessed with PolyPhen-2, version 2.2.2, HumVar option, and FATHMM version 2.3 with the prediction algorithm weighted for human mutations and disease ontology as the option for phenotypic associations. Nucleotide numbering according to the coding sequence of the *TPM3* cDNA reference sequence NM_152263.3. The first two ATG codons in the primary cDNA were both included according to the Human Genome Variation Society recommendations. Amino acid coordinates are provided relative to NP_689476.2; the ATG translation initiation codon is codon 1.

NM, nemaline myopathy; Cap, cap myopathy; CFTD, congenital fiber-type disproportion; CM, undefined congenital myopathy; spl, splice-site mutation; #, gain-of-function mutation resulting in hypercontractile phenotypes; M, male; F, female.

Table 3Summary of Clinical Findings in *TPM2* and *TPM3*

Clinical observation	<i>TPM2</i>	<i>TPM3</i>
Age of onset	Majority perinatally or in infancy, small number in childhood and few in adulthood	Majority perinatally or in infancy, small number in childhood and few in adulthood
Distal arthrogrypos is	Nine patients	None
Fiber size disproportion	29 families	35 families
NM	Milder presentation (10 NM cases, one adult onset)	More severe presentation (10 NM cases, four severe)
Patients with hypercontractile molecular phenotypes have more often contractures than others	Hypercontractile: contractures of limb joints (18/19) and jaw (6/19)	Hypercontractile: contractures of limb joints (18/19) and jaw (6/19)
	Others: contractures of limb joints (2/22) and jaw (1/22)	Others: contractures of limb joints (2/22) and jaw (1/22)

Summary of clinical findings in *TPM2* and *TPM3*.

NM, nemaline myopathy.